RAPID COMMUNICATION

Effect of fluoxetine on depression-induced changes in the expression of vasoactive intestinal polypeptide and corticotrophin releasing factor in rat duodenum

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Abstract

AIM: To investigate changes in vasoactive intestinal polypeptide (VIP) and corticotrophin releasing factor (CRF) in the plasma and duodenum of chronic stress-induced depressed rats and the effects of fluoxetine hydrochloride (fluoxetine) treatment on depression-induced changes in VIP and CRF.

METHODS: A Sprague-Dawley rat model of chronic stress-induced depression was produced. Thirty experimental rats were randomly divided into the following groups: control group, saline-treated depressed group, and fluoxetine-treated depressed group. Open-field testing was performed to assess the rats' behavior. VIP and CRF levels in plasma were measured by ELISA. Immunofluorescence techniques combined with laser scanning confocal microscopy (LSCM) were used to investigate VIP and CRF expression in the duodenum.

RESULTS: The open-field behavior, both crossing and rearing, of depression model rats, decreased significantly compared with those of normal control rats over 5 min. Defecation times increased significantly. Compared to the control group, FITC fluorescence of duodenal CRF expression and plasma CRF levels in the depressed rats increased significantly (fluorescence intensity of duodenal CRF: 11.82 ± 2.54 vs 25.17 ± 4.63 ; plasma CRF: 11.82 ± 2.54 ng/L vs 25.17 ± 4.63 ng/L, $P < 0.01$), whereas duodenal VIP expression and plasma VIP levels decreased significantly (fluorescence intensity of duodenal VIP: 67.37 ± 18.90 vs 44.51 ± 16.37 ; plasma

VIP: 67.37 ± 18.90 ng/L vs 44.51 ± 16.37 ng/L, $P < 0.01$). Fluoxetine improved depressed behavior, increased VIP expression and decreased CRF expression in plasma and the duodenal tissue of depressed rats.

CONCLUSION: Chronic stress can induce injury to the duodenum, accompanied by increasing CRF and decreasing VIP in the plasma and duodenum. Treatment with fluoxetine can ameliorate pathological changes in the duodenum of depressed rats, which suggests that antidepressants are an effective therapeutic agent for some duodenal diseases caused by chronic stress. VIP is a potential therapeutic strategy.

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Key words: Depression; Plasma; Duodenum; Rat; Vasoactive intestinal polypeptide; Corticotrophin releasing factor; Fluoxetine hydrochloride

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INTRODUCTION

In clinical studies, it has become clear that psychological factors, especially anxiety and depression, play an important role in gastrointestinal diseases by precipitating exacerbation of symptoms^[1,2]. Several studies have shown that the prevalence of chronic stress disorders in patients with gastrointestinal symptoms is about 60%-85%^[3,4]. Stress often worsens the symptoms of gastrointestinal diseases, which might be explained by altered neuroendocrine and visceral sensory responses to stress^[5].

In recent years, along with extensive research on the enteric nerve system (ENS), increasing evidence shows that peptidergic neurotransmitters could regulate gastrointestinal diseases. Vasoactive intestinal peptide (VIP), a 28-amino acid peptide, was first discovered, isolated, and purified from porcine intestinal extracts^[6]. It was also found in submucous and myenteric plexuses,

as well as the central and peripheral nervous systems^[7]. It is now recognized as a major neuropeptide in the brain and gut, with functions ranging from neurotransmission to neuromodulation with neurotrophic properties. Corticotrophin releasing factor (CRF) is a 41 amino-acid peptide which stimulates adrenocorticotrophic hormone (ACTH) secretion. Some data strongly suggest that CRF plays an important role in the pathophysiology of gastrointestinal diseases and electrophysiological properties of the brain during visceral perception^[8]. Patients with gastrointestinal diseases may have a higher tone of corticotropin-releasing hormone (CRH) in the brain. In common, both central and peripheral nervous pathways are involved in the release of gastrointestinal hormones due to psychological stress, thus modulating gastrointestinal motility^[9]. A large body of evidence derived from experiments suggests that CRF can accelerate small intestine transit, while VIP can inhibit it^[10].

Fluoxetine is a SSRI (selective serotonin re-uptake inhibitor), which are a class of antidepressants used in the treatment of depression and anxiety disorders. SSRIs increase the extracellular expression of the neurotransmitter serotonin by inhibiting its re-uptake into the presynaptic cell. Serotonin is also involved in the regulation of carbohydrate metabolism. Few analyses of the role of SSRIs in treating depression have covered the effects on carbohydrate metabolism from intervening in serotonin handling by the body. Studies have suggested that SSRIs may promote the growth of new neural pathways or neurogenesis^[11]. Also, SSRIs may protect against neurotoxicity caused by other compounds as well as from depression itself. Recent studies have shown that pro-inflammatory cytokine processes occur during depression in addition to somatic disease, and it is possible that symptoms manifested in these psychiatric illnesses are being attenuated by the pharmacological effects of antidepressants on the immune system^[12]. SSRIs have been shown to be immunomodulatory and anti-inflammatory against pro-inflammatory cytokine processes^[13,14].

However, there has been no report so far concerning the changes in VIP and CRF aroused by depression in plasma and duodenal tissue, and the effect of antidepressants on the duodenum. Therefore, we devised a rat depression model and observed the levels of VIP and CRF in the plasma and duodenum, and the effect of fluoxetine on the duodenum of depressed rats.

MATERIALS AND METHODS

Animals

Forty healthy male Sprague-Dawley rats, weighing 250 ± 30 g, from the Animal Center, Academy of Hubei Preventive Medical Sciences, were employed in the present study. The animals were fed standard rat chow, allowed access to tap water and were acclimated to their surroundings for 1 wk prior to the experiments. After this period, 30 rats were selected according to their open-field behavior.

Reagents

FITC (Fluorescein isothiocyanate)-conjugated goat anti-

rabbit IgG, VIP and CRF rabbit anti- mouse antibodies were purchased from Sigma Co., USA. Fluoxetine hydrochloride capsules were purchased from Lilly Co. Ltd., and ELISA kits were purchased from Beijing SUNBIO Biological Technology Co. Ltd. Other reagents used in the study were all of analytical grade.

Experimental protocols (preparation of the rat depression model treated with saline or fluoxetine)

A rat model of chronic stress-induced depression was established^[15-17]. The rats received a variety of stressors for 21 d, including tail nip for 1 min, cold water swimming at 4°C for 5 min, heat stress at 45°C for 5 min, water deprivation for 24 h, food deprivation for 24 h, 12-h inverted light/dark cycle (7:00 a.m. lights off, 7:00 p.m. lights on), paw electric shock (electric current 1.0 mA 10 s, every 1 min, lasting for 10 s, 30 times), *etc.* Stressors were administered throughout the experiment, could occur at any time of day (or night), and were each applied for a period of between 8 and 24 h. Their sequence was at random in order to be completely unpredictable to the animal. The animals were randomly divided into three groups (10 rats per group): model control, saline + chronic stress-induced, fluoxetine + chronic stress-induced therapy group. The depressed animals were treated with normal saline or fluoxetine (10 mg/kg) by stomach, (once a day, from the 24 h after the depressed model was established until the end of the experiment). A normal control group of rats (10 rats) without receiving any stress was included and housed in a separate room; food and water were freely available in their home cage.

Open field test (OFT)

The open-field test was designed to measure the reaction of rats to a novel environment. In this test, rats were individually placed in the center of a square, wooden, white-colored open-field box with 36 squares measuring 10 cm × 10 cm each. Their activity was assessed for 5 min. The number of squares from which rats crawled out was the total number of crossings. The number of occasions on which the animals stood on their hind legs was the total number of rearings. Defecation times were counted every 5 min. Each rat was housed in one cage and fasted before sucrose intake testing, after which 10 g/L sucrose solution consumption in 24 h was examined.

Assessment of duodenal histological damage

Duodenal tissue was sampled for a variety of determinations after the rats were anesthetized with 200 d/L urethane. Duodenal tissue was fixed in 4% paraformaldehyde, dehydrated, embedded in paraffin, sectioned in 4 μm thick sections, and stained with haematoxylin and eosin. The criteria of the histological score used was a previously validated scoring system from 0 to 4 that depends on the number and size of ulcers as well as the presence or absence of adhesions^[18,19]: (1) the infiltration of acute inflammatory cells: 0 = no, 1 = mild increasing, 2 = severe increasing; (2) the infiltration of chronic inflammatory cells: 0 = no, 1 = mild increasing, 2 = severe increasing; (3) the deposition of fibroin protein: 0 = negative, 1 = positive; (4) submucosa edema: 0 =

Table 1 Open-field activities and sucrose intake test of rats ($n = 10$, mean $\pm$ SD)

Group	Crossing/5 min	Rearing/5 min	Defecation/5 min	Sucrose intake mL/24 h
Control	133.00 $\pm$ 11.309	28.53 $\pm$ 10.22	3.90 $\pm$ 0.57	36.18 $\pm$ 10.24
Saline + depressed	51.80 $\pm$ 7.441 ^b	11.10 $\pm$ 5.18 ^b	6.80 $\pm$ 0.57 ^b	8.95 $\pm$ 7.39 ^b
FH + depressed	76.60 $\pm$ 3.534 ^d	15.58 $\pm$ 7.367 ^d	5.10 $\pm$ 0.43 ^d	18.10 $\pm$ 6.43 ^d

FH: Fluoxetine hydrochloride. ^b $P < 0.01$ vs control group; ^d $P < 0.01$ vs saline-treated depressed group.

Figure 1 Haematoxylin and eosin staining of duodenum tissues. **A:** No damage in the normal control group (HE, $\times 200$); **B:** Histological changes in the FH + depressed group (HE, $\times 200$); **C:** Histological changes in the saline + depressed group (HE, $\times 200$).

none, 1 = patchy-like, 2 = fusion-like; (5) epithelial necrosis: 0 = no, 1 = limiting, 2 = widening; (6) epithelial ulcers: 0 = negative, 1 = positive. The ulceration, inflammation, lesion and fibrosis were scored and put together as a result ranging between the minimum of 0 and maximum of 10.

Measurement of plasma VIP and CRF

Immediately after the rats were sacrificed, blood samples were collected into chilled tubes containing 0.3 μ L ethylenediamine tetraacetic acid (EDTA) and 1000 KIU aprotinin. Blood samples were immediately centrifuged at 3500 r/min at 4°C for 10 min. The supernatant was aspirated and stored at -70°C until analysis. VIP and CRF were determined by enzyme linked immunosorbent assays (ELISA) according to the manufacturer's instructions.

Detection of duodenal VIP, CRF expression

Duodenal tissue was fixed in 4% paraformaldehyde for 4 h. One hundred micron sections from the primary tissue were employed in the fluorescent immunohistochemical analysis, which used rabbit anti-rat VIP/CRF antibody, diluted 1:500 in phosphate-buffered saline (PBS). The staining procedure was as follows: (1) the sections were washed in PBS, then pretreated with 0.25% Triton X-100 for 30 min at 37°C and rinsed in PBS; (2) incubation for 12 h at 4°C in a 1:500 dilution of the primary antibody of VIP /CRF in PBS; (3) incubation with 1:100 diluted secondary antibodies (FITC -conjugated goat anti-rabbit IgG) in PBS for 30 min at 37°C. The sections were washed three times for 10 min after incubation steps 1 to 3, respectively, and were finally mounted in 50 g/L glycerin.

Detection was carried out according to the kit instructions (Leica SP2 TCS AOBS made in Germany). The specimens were excited with a laser beam at a wavelength of 488 nm (FITC). Five visual fields in three sections of each tissue were randomly selected and observed under a laser scanning confocal microscope (LSCM) and analyzed with a Leica Q500IW image analysis system in terms of FITC fluorescent intensity. This study

recorded the relative value of fluorescence intensity for the expression of VIP and CRF.

Statistical analysis

The data were expressed as the mean $\pm$ SD and analyzed with SPSS 11.5 statistic software. Statistical analysis was performed by using one-way ANOVA and Student-Newman-Keuls test for multiple comparisons. A P value less than 0.05 was considered statistically significant.

RESULTS

Open-field behavior of depression model rats (both crossing and rearing), was significantly decreased compared with that of the normal control rats (Table 1, $P < 0.01$). Defecation times significantly increased. The consumption of 10 g/L sucrose solution significantly decreased compared with that of the normal control (Table 1, $P < 0.01$). Treatment with fluoxetine hydrochloride (FH) significantly attenuated these effects.

Histological evaluation of the duodenum

No histological damage was seen in the normal control group. Rats with chronic stress-induced duodenitis showed neutrophil, macrophage, lymphocyte and eosinophil infiltration in the mucosa and submucosa. Ulceration and mucosal damage was obvious. Treatment with fluoxetine significantly attenuated the extent and severity of the histological signs. Damage scores of duodenum tissues were as follows: control: 0.39 ± 0.51 ; saline plus depressed: 7.46 ± 2.14 ; and FH plus depressed: 4.81 ± 1.37 (Figure 1).

VIP and CRF concentrations in plasma

Plasma VIP levels showed a significant difference among the three groups (Table 2, $P < 0.01$). VIP levels were higher in control and fluoxetine plus depression groups; however, they decreased significantly in the depressed group.

Plasma CRF levels showed a significant difference

Figure 2 Duodenal tissue staining procedure included: (1) pretreatment with 0.25% Triton X-100; (2) incubation in the primary rabbit anti-rat antibody of VIP/CRF; (3) incubation with secondary antibodies (FITC-conjugated goat anti-rabbit IgG). Expression of FITC-labeled VIP/CRF (green). **A:** Expression of VIP in the control group; **B:** Expression of VIP in the depressed group; **C:** Expression of VIP in the FH + depressed group; **D:** Expression of CRF in the control group; **E:** Expression of CRF in the depressed group; **F:** Expression of CRF in the FH + depressed group.

Table 2 Changes of levels of VIP/CRF in plasma ($n = 10$, mean $\pm$ SD)

Group	VIP in Plasma (ng/L)	CRF in Plasma (ng/L)
Control	67.37 $\pm$ 18.90	11.82 $\pm$ 2.54
Saline + depressed	44.51 $\pm$ 16.37 ^b	25.17 $\pm$ 4.63 ^b
FH + depressed	60.86 $\pm$ 19.27 ^d	17.05 $\pm$ 3.69 ^d

^b $P < 0.01$ vs control group; ^d $P < 0.01$ vs saline-treated depressed group.

among the three groups (Table 2, $P < 0.01$). CRF levels were lower in control and fluoxetine plus depression groups, but increased significantly in the depressed group.

VIP and CRF alterations and the effects of fluoxetine on the content of VIP and CRF in duodenum tissue of depression model rats

Compared with that of normal control rats, the average fluorescence intensity of duodenal CRF increased significantly, while the average fluorescence intensity of duodenal VIP decreased significantly in chronic stress-induced depressed rats (Table 3, $P < 0.01$). Furthermore, a significant improvement of the elevated duodenal VIP content and the significant reduction of duodenal CRF content were observed in animals treated with fluoxetine (Figure 2, Table 3, $P < 0.01$).

DISCUSSION

We found that experimental rats had almost all demonstrable symptoms of depression, consistent with the classic and mature model of depression^[16,20]. Our results showed that both crossing and rearing behavior of depressed rats over five minutes significantly decreased compared with that of the normal control rats (Table 1, $P < 0.01$). Defecation times significantly increased. The consumption of 10 g/L sucrose solution significantly decreased compared with that of the normal control.

Table 3 The average fluorescence intensity analysis of VIP and CRF alterations in duodenum of depression model rats ($n = 10$, mean $\pm$ SD)

Group	Fluorescence intensity of VIP	Fluorescence intensity of CRF
Control	36.28 $\pm$ 17.16	10.87 $\pm$ 9.28
Saline + depressed	19.07 $\pm$ 13.84 ^b	50.83 $\pm$ 24.66 ^b
FH + depressed	28.29 $\pm$ 15.02 ^d	29.18 $\pm$ 17.34 ^d

FH: Fluoxetine hydrochloride; VIP: Vasoactive intestinal polypeptide; CRF: Corticotrophin releasing factor. ^b $P < 0.01$ vs control group; ^d $P < 0.01$ vs saline-treated depressed group.

Crossing reflected the degree of animal activity, rearing reflected the degree of curiosity to the novel surroundings, defecation times responded to intestinal function, and sucrose intake tests reflected the animal's response to rewards^[21,22]. The chronic stressors caused a generalized decrease in action and responsiveness to rewards, and a functional gastrointestinal disorder. The behavioral changes of the depressed rat were reversed by chronic treatment with fluoxetine hydrochloride (a type of antidepressant).

On the other hand, rats with chronic stress-induced depression showed significant histological damage from duodenitis. For example, a number of neutrophils, macrophages, lymphocytes and eosinophils were found in the mucosa and submucosa; ulceration and mucosal damage could also be observed. No change was seen in the normal control group, and treatment with fluoxetine hydrochloride significantly attenuated the extent and severity of the histological signs. The antidepressant fluoxetine hydrochloride inhibited the extent of inflammation, prevented mucosa injury, minimized the ulceration area, and alleviated the duodenitis seen in the depressed animals.

In this study, we also found that there were different changes in VIP and CRF in the depressed rats' duodenum and plasma. The average fluorescence intensity of duodenal CRF expression of the depression model rats

increased significantly compared with that of the normal control rats, whereas that of duodenal VIP expression decreased significantly.

Brain gut peptides (BGPs) are distributed extensively in the brain and the gastrointestinal tract. Studies have demonstrated that some BGPs including VIP and CRF participate in gastrointestinal motility, secretion and absorption^[23]. Several investigations have found that basal CRF levels have increased significantly during stress in patients. Because the gut and the brain are highly integrated and communicate in a bidirectional fashion largely through the ANS and HPA axis, patients also responded with higher expression of ACTH during stress and had higher basal expression of noradrenalin than the normal group. Stress induced exaggeration of the neuroendocrine response and visceral perceptual alterations occur during and after stress by CRF^[6]. On the other hand, some studies have indicated that VIP participated in the modulatory effect of drugs on gastrointestinal motility and played an important role in gastrointestinal disorders caused by psychological stress^[24]. It had been demonstrated that stress-induced plasma VIP expression decreased gastrointestinal transit disorder beyond a certain intensity range of stress. VIP had potent protective activity against sepsis and increased the survival rate of septic animals^[25]. In this study, the significant increase in the expression of CRF possibly suggests that depression could induce an inflammatory response of the duodenum by releasing CRF in rats. The effect of VIP on inflammatory cells could be an additional important mechanism of its potent protective activity on chronic stress-induced duodenitis. The results of our studies suggest that gastrointestinal motility disorders during psychological stress may be partially mediated by release of VIP and CRF.

Our results also show that an antidepressant plays an important role in decreasing symptoms in depressed rats. The behavioral changes of depressed rats were reversed by chronic treatment with fluoxetine. Treatment with fluoxetine significantly attenuated the extent and severity of the histological signs. Fluoxetine at the therapeutic dose of 10 mg/kg was effective in decreasing the expression of CRF and increasing the expression of VIP in the duodenum of depressed rats. Some data has shown that antidepressants may adjust other brain gut peptides or unknown factors, and thus ameliorate the damage of chronic stress-induced gastrointestinal disorders^[26-28]. Future serotogenic antidepressants may be made to specifically target the immune system by either blocking the actions of pro-inflammatory cytokines or increasing the production of anti-inflammatory cytokines^[29,30].

In summary, brain-gut interaction and psychological factors altered not only the pathology of brain tissue, but also duodenal tissue. The results of our study show that depression can induce injury to the duodenum accompanied by increasing CRF and decreasing VIP. Treatment with fluoxetine can ameliorate pathological changes in the duodenum in depressed rats, suggesting that SSRIs are an effective therapeutic agent for some duodenum diseases caused by psychological factors. We suggest that VIP prevention of inflammatory cell reactivity could be a potential therapeutic strategy for chronic stress-induced gastrointestinal disorders.

COMMENTS

Background

In clinical studies, it has become clear that depression plays an important role in gastrointestinal diseases by precipitating exacerbation of symptoms. Stress often worsens the symptoms of gastrointestinal diseases.

Research frontiers

Some data strongly suggested that corticotrophin releasing factor(CRF) and vasoactive intestinal peptide(VIP) played important roles in pathophysiology of gastrointestinal diseases. Selective serotonin reuptake inhibitors (SSRI's) have been shown to be immunomodulatory and anti-inflammatory against pro-inflammatory cytokine processes.

Innovations and breakthroughs

We set up a rat depression model, and observed the level of VIP and CRF of plasma and duodenum and the effect of fluoxetine on duodenum of the depressed rats.

Applications

The authors of the present study showed that chronic stress can induce injury to the duodenum. This was accompanied by an increase in immunofluorescence staining for CRF and a decrease in immunofluorescence staining for VIP. Treatment with the serotonin reuptake inhibitor, fluoxetine, reversed these changes and in addition reversed the behavioral changes of depressed rats.

Terminology

Vasoactive intestinal peptide (VIP) was found in submucous and myenteric plexus, central and peripheral nervous systems. It is now recognized as a major neuropeptide in the brain and gut. Corticotrophin-releasing factor (CRF) stimulates adrenocorticotrophic hormone (ACTH) secretion and plays an important role in the pathophysiology of gastrointestinal diseases.

Peer review

The authors of the present study showed that chronic stress can induce injury to the duodenum. This was accompanied by an increase in immunofluorescence staining for CRF and a decrease in immunofluorescence staining for VIP. Treatment with the serotonin reuptake inhibitor, fluoxetine, reversed these changes but in addition reversed the behavioral changes of depressed rats.

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